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Seminars in Virology

The Mystique of the Herpesviruses

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Probing the biologic mystique of the herpesvirus family, we find elements of antigenic similarity, the capacity of virions to persist within our cells in complete form, and the potential at some later time for these virions to undergo renewed replication. A venereal mode of transmission from one host to another may be characteristic of some of these viruses. Similar patterns of illness may be produced by several of the herpesviruses. Within the cellular framework of inflammation and necrosis which infective virus may evoke in the host, there may also reside the capability of neoplastic transformation.

THE HERPESVIRUS FAMILY has evolved from what seemed to be very divergent origins ten to twenty years ago. All members of this group of viruses share some antigenic relationship, and to the electron microscopist they present similar morphologic characteristics of size and shape. Most crucial for the human host, they share biologic characteristics. Primary infections are often silent and the more unique expressions of illness with these viruses may unfold upon reinfection or reactivated infection. Virus may persist in incomplete resting or subviral form. Some of the members of this diffuse family of viruses retain a venereal mode of transmission and some indeed may be oncogenic. We wish to illustrate the convergent relationships which exist within the herpesvirus group by reviewing each member virus and the biologic problems which these viruses pose for man.

Herpes Simplex Virus, Type 1 and Type 2

The relationship of herpes simplex virus (HSV) type 2 to cancer of the cervix is intriguing and implicates not only the oncogenic potential of this virus but also its apparent mode of venereal transmission. The provocative epidemiologic studies of Rawls and his colleagues[1] have pointed out the striking difference in immunologic experience with HSV type 2 between a control population and a group of women with cervical carcinoma. The uniqueness of this serologic observation is ampli-

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fied by the fact that members of both groups had had equivalent previous immunologic experience with the oropharyngeal serotype of HSV type 1. Naib and coworkers[2] observed that 7 percent of women with obvious genital HSV type 2 infection had *in situ* carcinoma of the cervix. Only 0.6 percent of women who had not had genital herpesvirus infection had a similar precancerous lesion. In further confirmation of the venereal mode of transmission, Nahmias et al[3] demonstrated that 3 percent of nuns had antibody to HSV type 2 whereas Rawls and coworkers[4] demonstrated that 43 percent of prostitutes had type 2 antibody. While the population begins to acquire antibody to type 1 virus at an early age, antibody to the type 2 virus appears at the time of pubescence and escalates strikingly during the sexually active years which follow.

Very recently Rapp and his colleagues[5] demonstrated that HSV type 2 is capable of transforming hamster embryo fibroblasts into neoplastic cells.

The biologic characteristics of HSV type 2 differ from those of type 1 strains. They are ordinarily genital strains, produce much larger pock lesions on the chorio-allantoic membrane of the embryonated chick, are more paralytogenic for adult rabbits, and are more likely to disseminate in a direct, non-viremic fashion to the central nervous system. Thus this serotype may be more neurovirulent than type 1 strains.

These biologic characteristics are of great importance to the physician who must deal with perinatal HSV infections. Nahmias, Alford, and Korones[6] reported that the great majority of isolates from neonatal infections are type 2. The illness afflicts mainly pre-term infants; the ratio of infected pre-term to term infants is 1:1.5. This observation is supported by our own studies, which indicate a more striking innate susceptibility of immature tissues to HSV replication.[7] The incidence of perinatal HSV infection is estimated to be 1 in 7,500 deliveries. Hence it might be anticipated that 120 cases will occur in the United States each year. We suspect that with heightened promiscuity and the element of venereal transmission the incidence of neonatal infections is far greater.

We understand the natural history of neonatal HSV infections in only a very superficial manner. When pathologic descriptions serve as the primary source of cognitive clinical information, one must assume that non-lethal cases have eluded the detection of clinicians. Although Nahmias and colleagues[6] suggested that infants with disseminated HSV infection almost uniformly die, one must wonder how often the clinician and the virologist fail to detect mild to moderate disseminated infection, with or without neurological involvement. If such children do not have oropharyngeal or cutaneous lesions, there is no way at present whereby one may make a diagnosis, and indeed many of these cases may survive without the availability of the precise biologic diagnosis ordinarily attained at the necropsy table. Nature suggests that we are dealing with only the tip of the iceberg when we consider that newborn infants with localized infection of the central nervous system, eye, skin, or oral cavity do not uniformly die and may recover health. As experience with the use of pyrimidine analogs for systemic antiviral chemotherapy continues to evolve, there must develop a solid perspective for the natural history of this illness if valid conclusions are to be drawn about chemotherapeutic efficacy. There is the obvious need for a nationwide collaborative study wherein the many medical centers which deal with these infants may coordinate methods of study and pool careful clinical and virologic observations.

The incidence of cervical-vaginal shedding of HSV type 2 is slightly less than 1 percent. This rate of shedding is to be contrasted with the 2 to 5 percent of our population which shed HSV type 1 in the oropharynx. We must determine whether a pregnant woman is more likely than the non-gravid to shed virus in her cervical-vaginal tissues. It will also be important to determine how the quantity of virus in the birth canal relates to subsequent mild or serious infection of the newborn.

Some very important points have been brought to light during recent years which have clarified our understanding about the traffic patterns of virus dissemination from the respiratory tract or the genital tract to neural tissue and in the opposite direction. It is known that fully mature virions may be distributed down the course of peripheral nerves either by streaming through the axoplasm or by cell to cell transmission within the Schwann sheath cells which surround the nerves. This fundamental phenomenon explains, of course, the occasional zosteriform morphologic feature of cutaneous HSV infection. Recently, Stevens and Cook,[8] with some elegant experiments, pointed out that HSV, probably in the form of non-encapsidated and non-enveloped genomic subvirion, may become latent in ganglion cells. Utilizing the innovative technique of ganglion tissue culture, these workers were able to unmask latent HSV particles within the ganglion tissue of experimental animals. Traditional virologic techniques failed to detect mature, complete virus.

Varicella-Zoster

Infections produced in man by varicella-zoster, a member of the herpesvirus family, represent the classic example of the biologic point developed above—namely, that primary infection, chickenpox, is followed by latency in neural tissue for many years only to re-express itself with the sharply different and very classic dermatomorphology of herpes zoster (shingles).

Cytomegalovirus

In the context of the exploration of the natural history of HSV infections in the neonate, it is illuminating to review our understanding of congenital cytomegalovirus (CMV) infection in 1972 and compare that with our understanding of this disease ten years before. It was 1962 when Weller and Hanshaw[9] described, in what is now considered one of the classics in the CMV literature, the full sweep of this infection in newborn infants. Clinicians considered this to be a rare illness characterized by severely damaged and stunted infants with hepatosplenomegaly, microcephaly, intracranial calcifications, thrombocytopenic purpura, hemolytic anemia, and severe neurologic impairment. At present we know that congenital CMV infection is indeed very common, occuring in perhaps 1 percent of all newborn infants.[10] Few of these infants present the expanded syndrome of devastating disease as was originally described. Rather, a majority are perfectly normal. A few may have only intrauterine retardation of growth. Emanuel and Kenny[11] described infants with only idiopathic hyperbilirubinemia. Although other investigators have not fully supported his observations, Hanshaw[12] has suggested that some infants and children affilicted as fetuses with CMV infection develop only otherwise unexplained microcephaly. Although congenital CMV infection does not seem to be as awesome a clinical problem as it was reported to be a decade ago, careful evaluation of these otherwise normal infants reveals that approximately 20 percent have incurred some neurologic damage and mental retardation.[13] Other isolated sequelae of congenital CMV infection include neonatal hepatitis and a surprising incidence of inguinal hernia.[14] Much as has been described with congenital rubella virus infection, CMV infected infants may be deaf and may also have the metaphyseal radiolucency of long bones which has been considered to be so characteristic of congenital rubella.[15,16] However, cardiovascular defects are not a prominent part of the clinical spectrum of congenital CMV infection.

There must be some reason for this remarkable incidence of infection, and Numazaki and his colleagues[17] have observed that during the course of gestation there is an increasing incidence in the rate of shed of this virus in the vaginal secretions of the pregnant woman. Although they were unable to detect vaginal shedding of CMV during the first trimester of pregnancy, they pointed out that 28 percent of women were shedding this virus by the end of gestation. Medearis and his colleagues,[18] in Pittsburgh, have also found a significant percentage of women shedding CMV in their vaginal secretions during pregnancy. Reynolds et al[19] have detected a similar striking increase in the shedding of virus from the cervix throughout the course of pregnancy. It cannot be concluded absolutely either that these maternal infections with CMV represent acquired illness or that they reflect reactivation of virus replication during the late phases of pregnancy. Careful serologic studies suggest that cervical-vaginal CMV shedding occurs as a reactivation phenomenon in that detectable levels of CMV complement-fixing antibody remain stationary throughout the period of gestation.[17,19]

Another intriguing component to congenital CMV infection is the possibility of venereal transmission. In one study the incidence of congenital infection was found to be 1 in 114 cases of unmarried women, in contrast to the incidence of congenital infection in married women of 1 in 700.[20]

An important new syndrome is that of CMV mononucleosis.[21] This illness is characterized by fever without the presence of pharyngitis and adenopathy which one anticipates in typical infectious mononucleosis. The heterophile antibody test is ordinarily negative but atypical lymphocytes are present. Very closely interlinked with CMV mononucleosis is the post-perfusion syndrome which has also been attributed to infection with CMV. The syndrome is an infectious mononucleosis-like illness with fever, splenomegaly and the presence of atypical lymphocytes, but a negative heterophile antibody test. This syndrome is associated with the transfusion of fresh blood, usually no more than two to three days old.

There are some observers who still believe that this post-perfusion syndrome represents reactivation of CMV replication within the host rather than the acquisition of virus via the leukocytes of fresh blood.[22] The incidence of the disease seems to relate clearly to the number of units of whole blood used during the surgical procedure. It has been observed that 5 percent of blood donors carry CMV in their leukocytes.[23] Indeed, Lang et al[24] have demonstrated that the elimination of leukocyte precludes the acquisition of infection by recipients. All of these facts would suggest that the post-perfusion syndrome represents an illness acquired as a result of the direct transfusion of virus into the host.

The intriguing experience of the Henles and coworkers[25] gives additional support for the notion that this illness is an acquired infection. They found that 36 percent of cases of post-perfusion syndrome were CMV in origin, so indicated by CMV antibody rise. It is amazing to note that of all the patients which they studied, 40 percent were CMV serosusceptible. Thus, their figures for attack rate correlate very closely with the segment of the patient population originally deemed to be susceptible. Only 8 percent of the post perfusion cases were caused by the Epstein-Barr virus (EBV). They found that 10 percent of the entire study group were susceptible to EBV, and so once again the great majority of susceptible persons incurred infection once exposed to the virus by transfusion.

Epstein-Barr Virus

Pathogenic relationships have been established between the Epstein-Barr virus (EBV) and infectious mononucleosis as well as Burkitt's lymphoma and nasopharyngeal carcinoma. The original observations at Yale University had the greatest impact on the establishment of an etiologic relationship between this virus and infectious mononucleosis.[26] It was observed that antibody-negative freshman students were the only students who acquired this infection at any time during the remainder of their collegiate years. Freshman who were antibody-positive at the outset did not acquire infectious mononucleosis. Therefore, much as in the post-perfusion syndrome, disease seemed to occur only in persons in whom susceptibility was demonstrated by the lack of protective antibody. Dr. James Grace[27] performed an important experiment when he transmitted the disease of infectious mononucleosis by the inoculation of EBV into a youngster with lymphatic leukemia. Recently, Shope et al[28] reproduced the disease in the rhesus monkey by introducing infected leukocytes. Serologic diagnosis, employing the EBV complement-fixing antibody assay, may be unrewarding in the evaluation of infectious mononucleosis. Antibody titers appear so quickly at the outset of symptomatic infection that it is difficult to demonstrate a significant four-fold rise during the early weeks of illness.

One of the great students of this disease has been Dr. Alfred Evans. Without the knowledge of a specific pathogen or the presence of virologic and serologic tools to carefully study such an illness, Evans[29] suggested many years ago that primary illness in the child was much different than that in the adult. Youngsters presented with fever and rash, perhaps splenomegaly and lymphadenopathy, and the appearance of atypical lymphocytes. The heterophile antibody tests were ordinarily negative. As the potential host aged through puberty into the early adult years, Evans documented the classic illness with fever, sore throat, splenomegaly, lymphadenopathy, atypical lymphocytes, and now the elaboration of heterophile antibody.

Now with the necessary virologic and serologic tools available, similar epidemiologic studies have been performed and they accurately confirm the earlier predictions and observations of Evans. Primary EBV infection in a very young child may produce nothing more than an undifferentiated febrile illness. During the early school years a further differentiated form of disease, with lymphadenopathy, splenomegaly and atypical lymphocytes, occurs. As Evans had anticipated, heterophile antibodies are usually negative. Careful seroepidemiological studies of EBV infection by Deinhardt and coworkers[30] demonstrated both in Chicago and in Alaska that the great majority of children and adults have antibody to this virus. This is surprising in light of the original observations of the Yale investigators which indicated that only 25 percent of beginning students at New Haven had antibody to this virus.

Recent observations by Hanshaw and his colleagues[31] provide continuing support for the taxonomic relationship of EBV, CMV and varicella-zoster virus within the entire herpesvirus family. These investigators detected macroblobulin (IGM) CMV antibody rise early in the course of illness with EBV infection. They were unable to detect complement-fixing CMV antibody response, ordinarily a 7s (IGG) antibody, in persons with documented EBV infectious mononucleosis. The same observation was made with varicella-zoster virus infection. Early in the course of chickenpox and shingles there appeared a rise of IgM CMV antibody without a concommitant rise of CMV complement-fixing antibody. Because IgM antibodies may reflect a more primitive and broad antigenic relationship existing among all of these herpesviruses, one might anticipate an early IgM antibody response to any of the other viruses within the family in the event of a primary illness. Rather than dual infection with two herpesviruses, it seems that infection with one herpesvirus calls forth a primitive, abbreviated immunologic response to other members of this virus family with which the host has had an antigenic experience in the past.

Some patients with adenopathy, splenomegaly, hepatomegaly, occasional rash, sore throat, a few atypical lymphocytes and negative heterophile antibody, but no EBV antibody rise, may reflect a unique response to infection with coxsackievirus B5.[32] Other patients with atypical lymphocytosis and fever alone may have CMV infection and the diagnosis may be elucidated by searching for CMV antibody. When confronted with infectious mononucleosis-like illness and its various manifestations, one should consider EBV, CMV, coxsackievirus B5, and also primary toxoplasmosis.

The post-perfusion syndrome associated with EBV is characterized by fever, splenomegaly, and atypical lymphocytes.[25] As was noted for CMV, 5 percent of blood donors have been shown to be carriers of EBV. EBV persists in lymphocytes and may mature and release from cells when cultured *in vitro,* a phenomenon quite similar to the fundamental cellular relationship of some of the other herpesviruses with neural tissue in the human host.

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